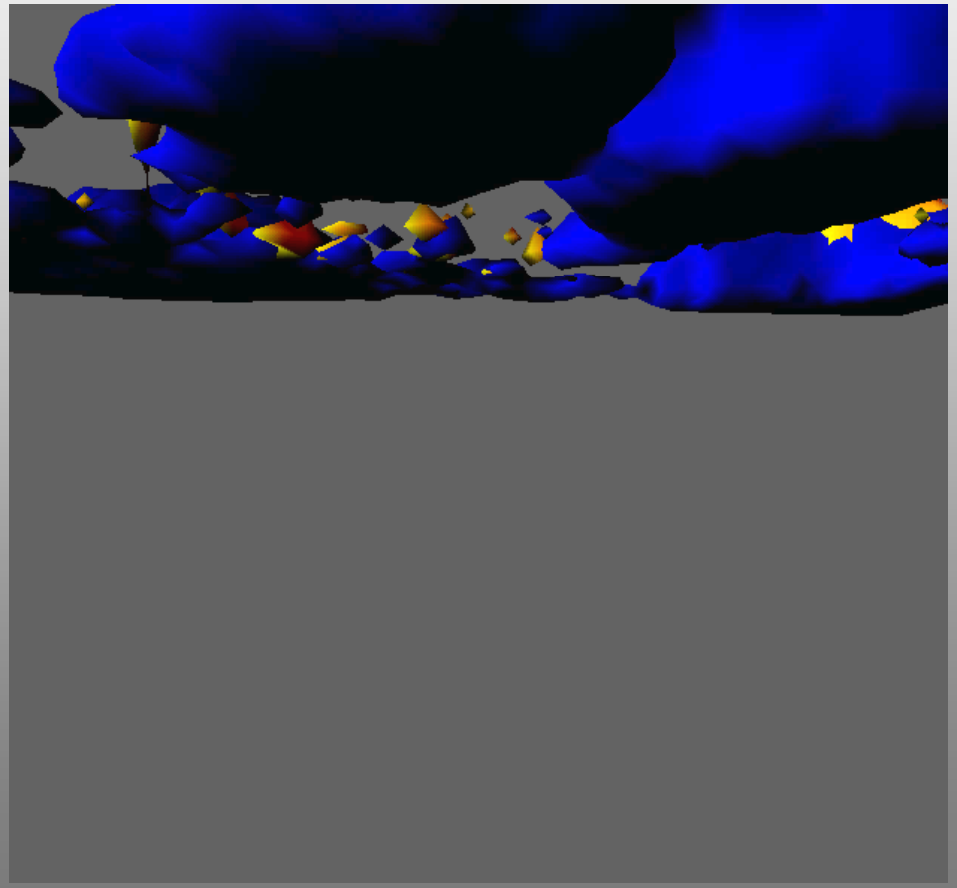
# Biofilms

## The Surface Associated Microbial World



# Bacterial biofilms are everywhere

**Biofilm: Bacteria attached to a surface encased in a matrix of extra-cellular polymeric substances (EPS)**

**99% of all bacteria in nature are enclosed in biofilms**

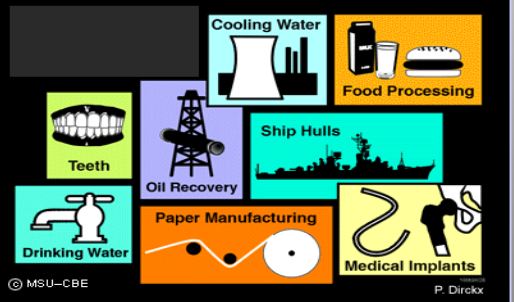
**From single cells to organized multi-cellular mature biofilms**

## Where do we find biofilms?

- Fresh-water reservoirs
- The sea
- Soil particles
- Teeth
- Water pipes, water tanks and fermentores
- On internal bio-surfaces of humans and animals
- In cystic fibrosis, on prosthetic devices, etc.
- On food
- Basically any place that supports life

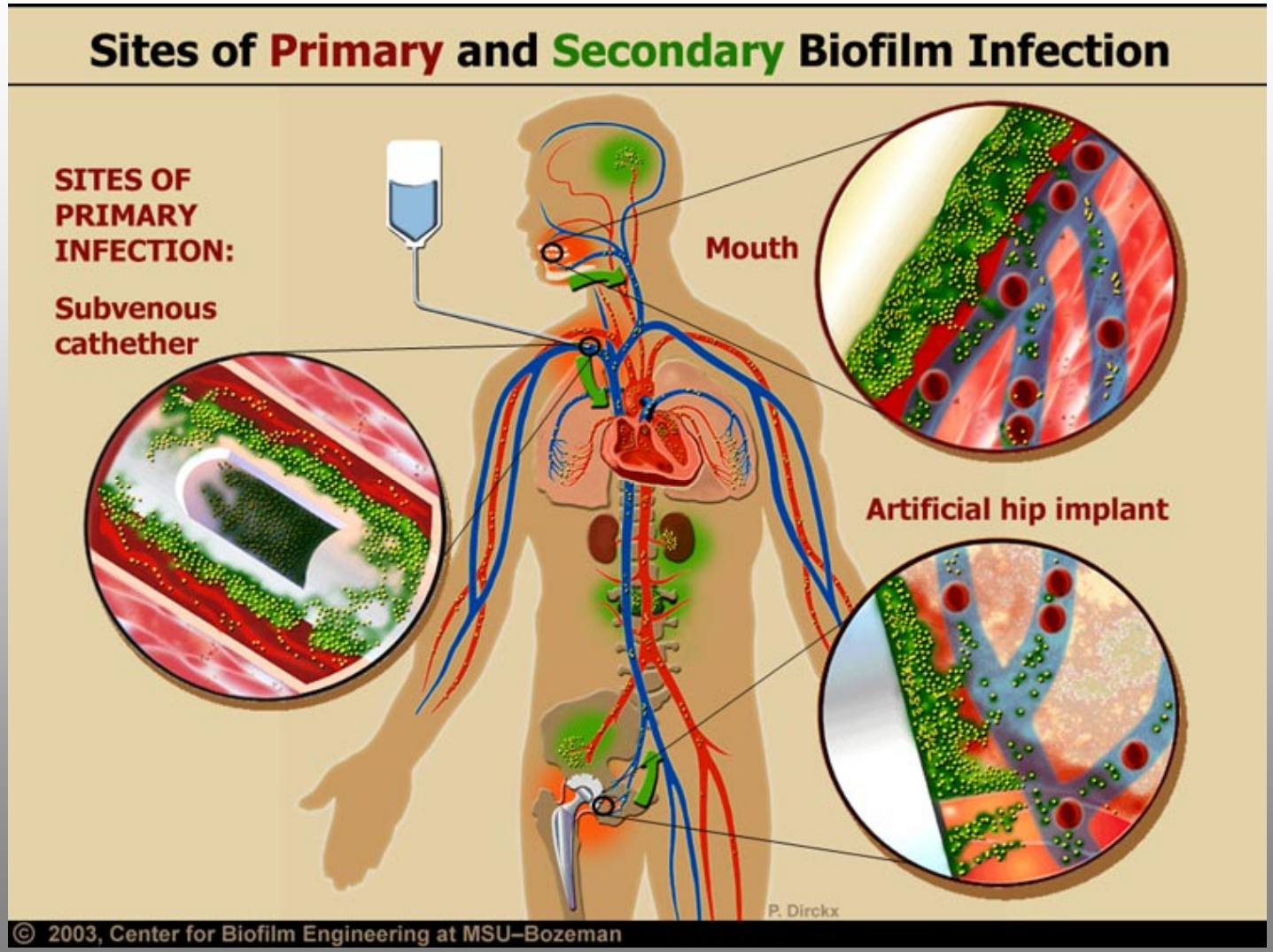
Jurassic oolithic microbial mat community with fungal networks (200 million years old)

**….AND IN THE LAB UNDER CONTROLLED CONDITIONS**



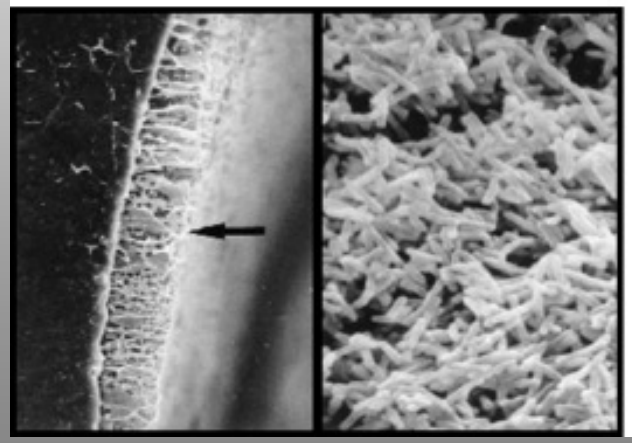

**In addition to the studies of bacteria in test tubes, it is important also to investigate the bacteria when growing as biofilms.**

**Why, because they here in many cases perform in a very different way**

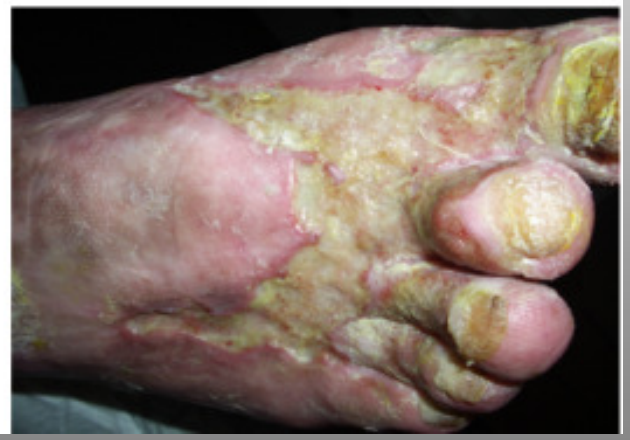

Sites of Primary and Secondary Biofilm Infection
SITES OF PRIMARY INFECTION:
Subvenous cathether
Mouth
Artificial hip implant
P. Dirckx
© 2003, Center for Biofilm Engineering at MSU–Bozeman

# Medical relevant biofilms



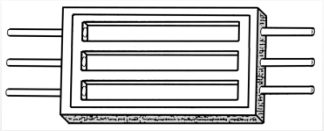


Catheter

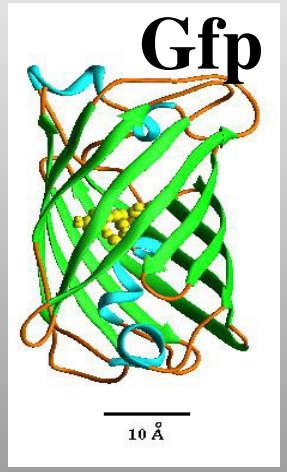


Chronic wounds

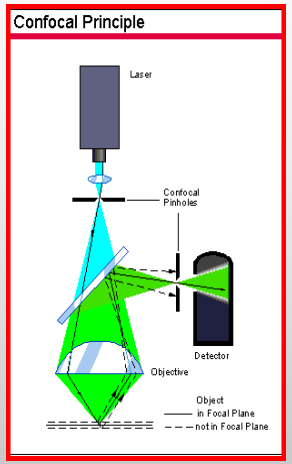
# The laboratory biofilm tool-box

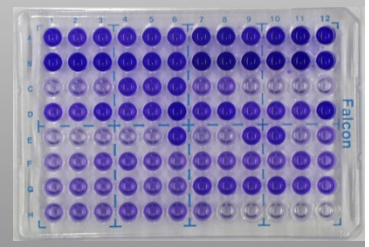


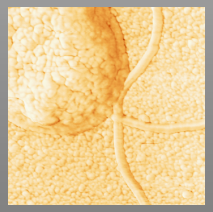
- Flow chamber system for biofilm cultivation
- GFP – Green Fluorescent Protein



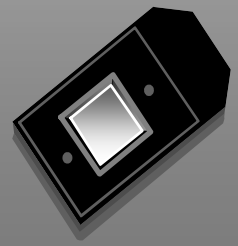

- SCLM – Scanning Confocal Laser Microscope
  - Atomic force microscopy and SEM
- Microtiter assay for biofilm formation
  - The robot and colony picker
- The Cell sorter (FACS)



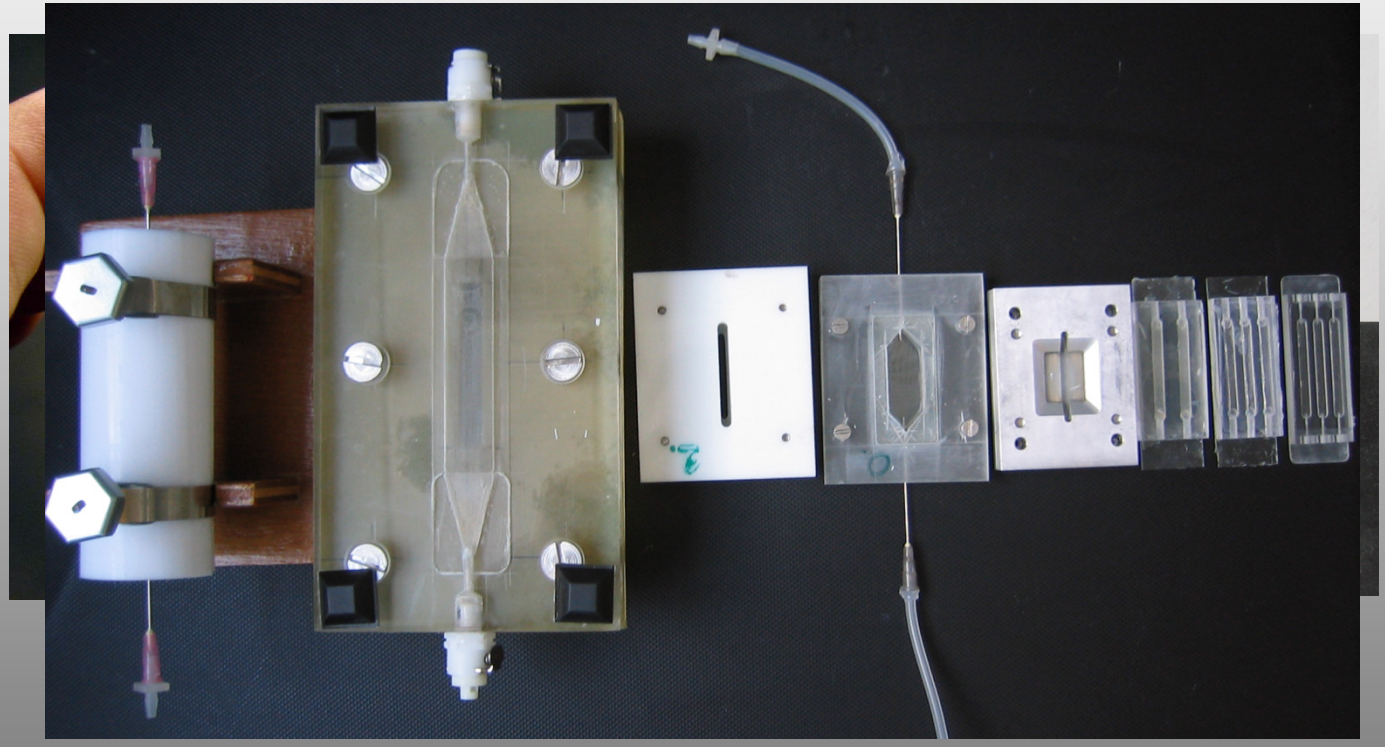



- Micro array analyses
- FISH – Fluorescent *In Situ* Hybridisation
- COMSTAT



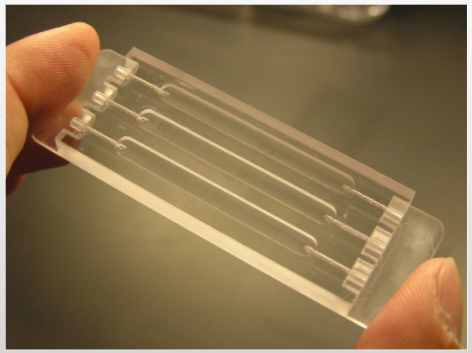
# The DTU flow cell system

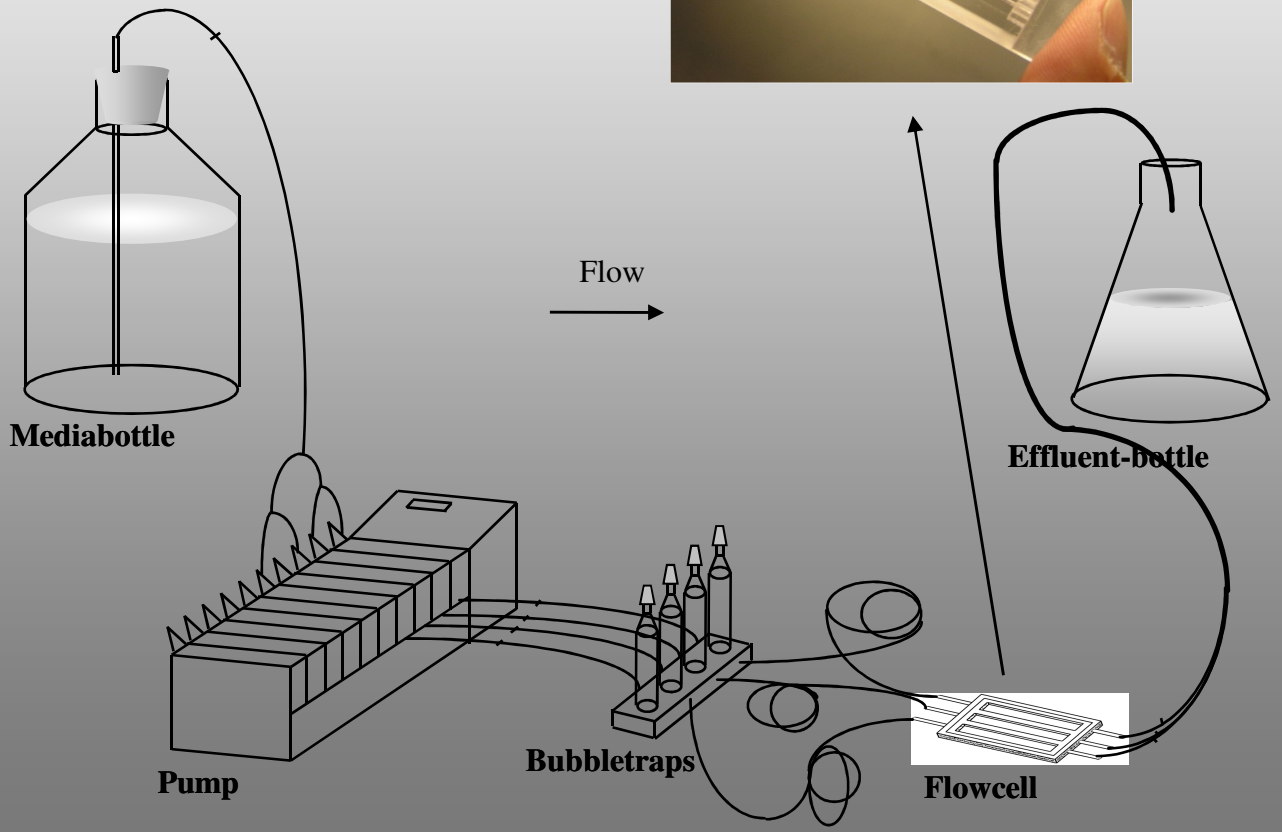


# The Flow Chamber System

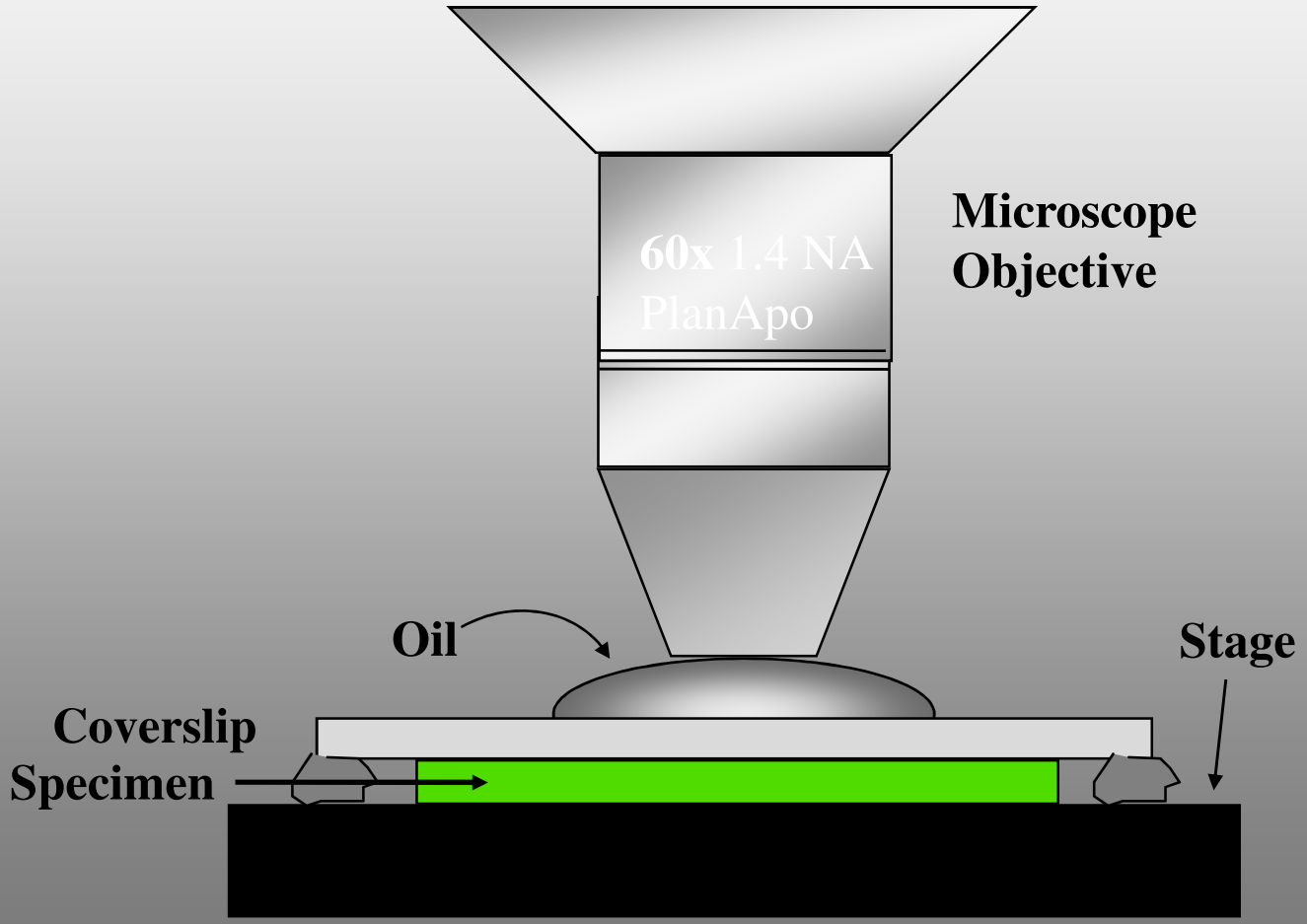
**The standard set-up at DTU for hydro-dynamic biofilm development.**

**The flow-cells are mountable directly on the Confocal Microscopes for in situ investigations (Zeiss LSM510 or Leica DMRXA)**





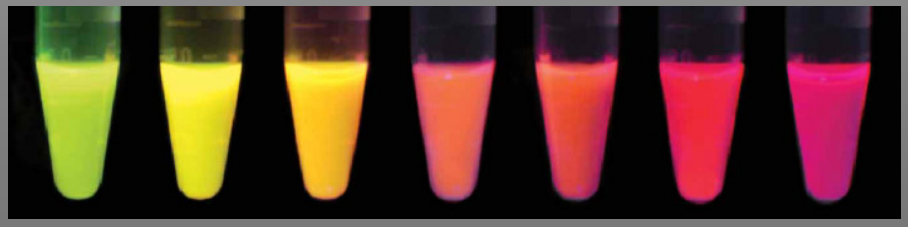
# Microscope Objectives

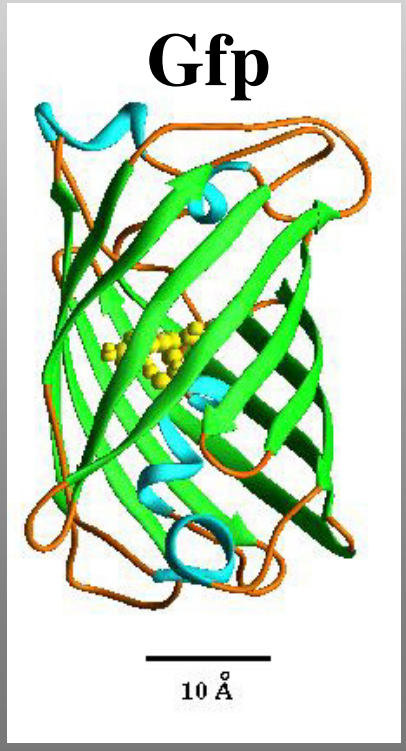


# Green fluorescent protein (Gfp)

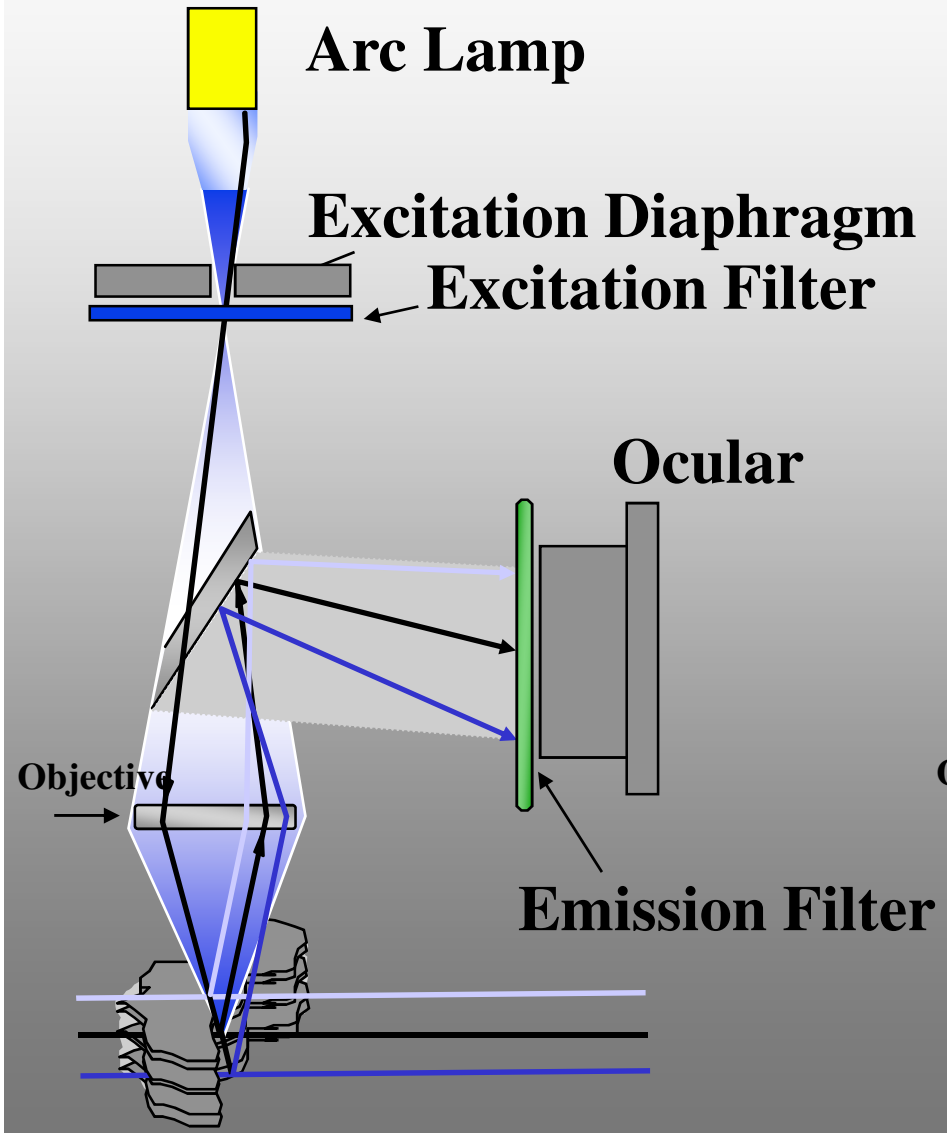
Tagging and reporter cassettes with Gfp, Yfp, Cfp, and Rfp genes are available

- Isolated from the jelly fish *Aequorea victoria*
- Excellent for studying gene expression in vivo
- Molecular reporter cassettes for visualization of e.g. substrates and growth activity
- Requires only oxygen to maturate
- Works in bacteria, yeast, plants and mammals

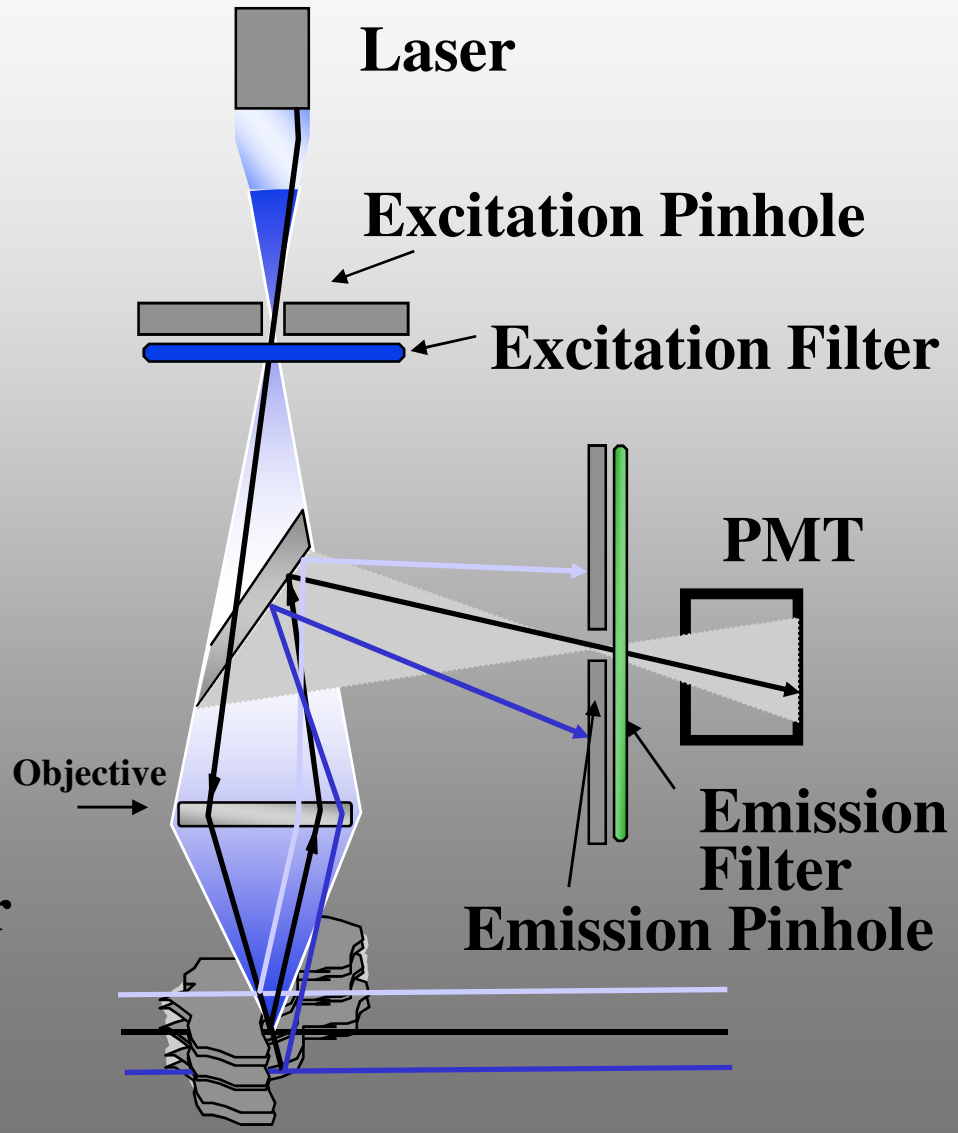





## Fluorescent Microscope



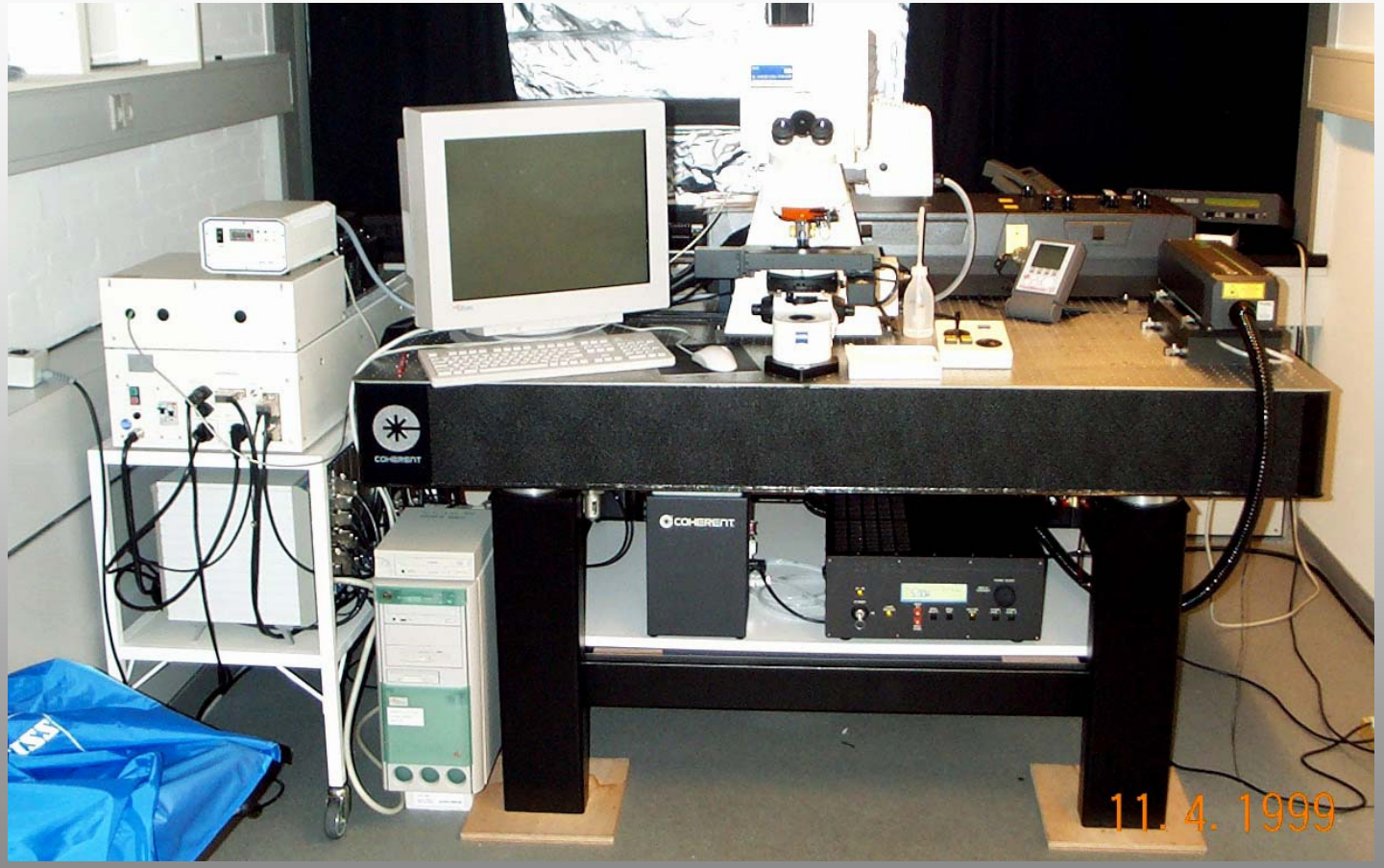

## Confocal Microscope

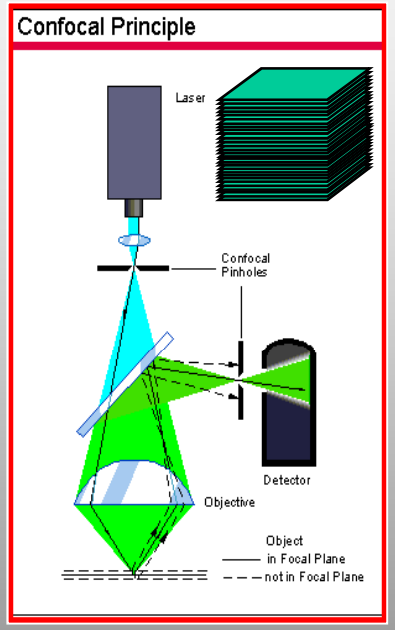


# Benefits of Confocal Microscopy

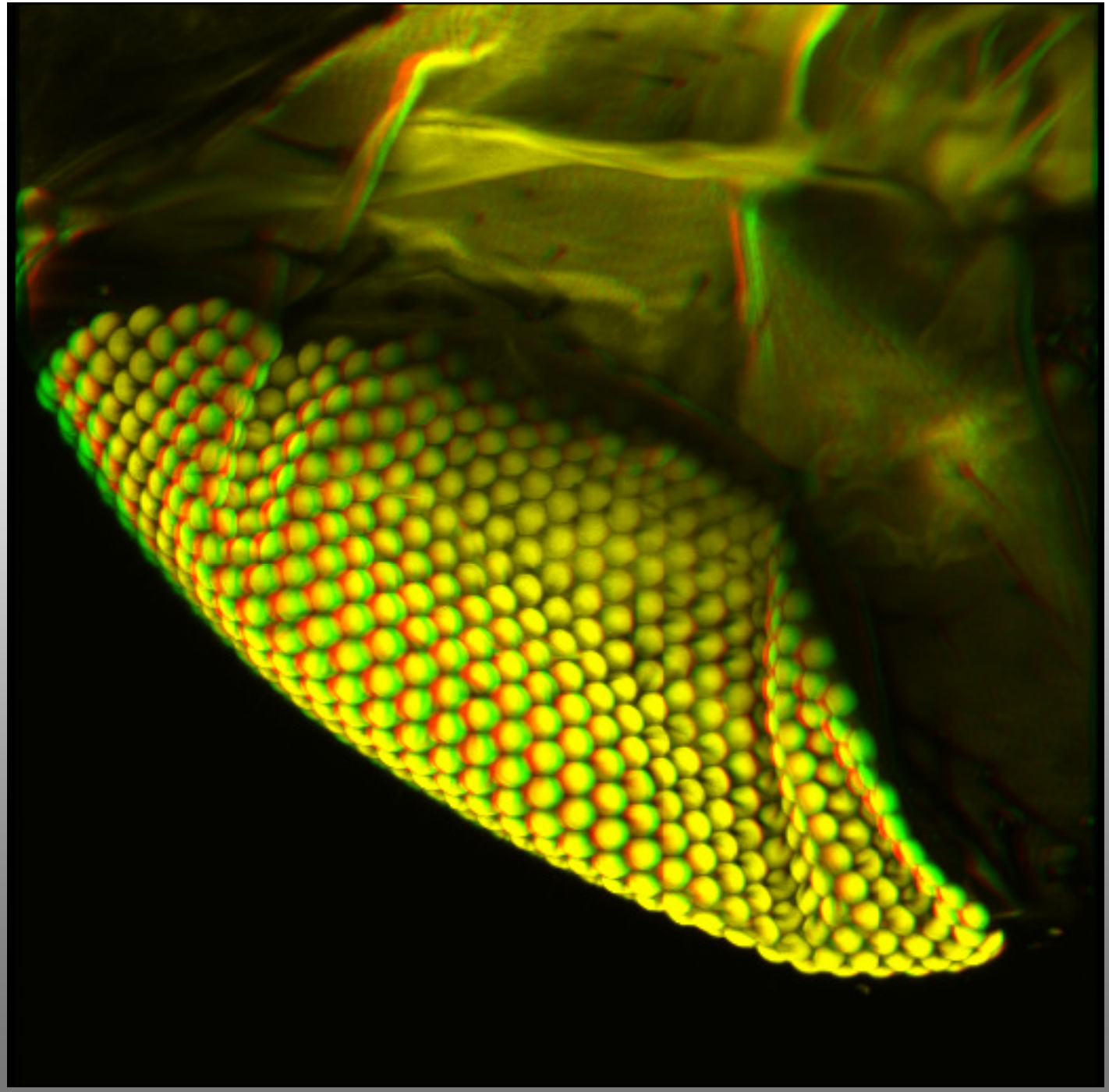
- Reduced blurring of the image from light scattering
- Increased effective resolution
- Improved signal to noise ratio
- Clear examination of thick specimens
- Z-axis scanning
- Magnification can be adjusted electronically

# Multiphoton facility on the Zeiss confocal




Confocal Principle
Laser
Confocal Pinholes
Detector
Objective
Object
in Focal Plane
not in Focal Plane

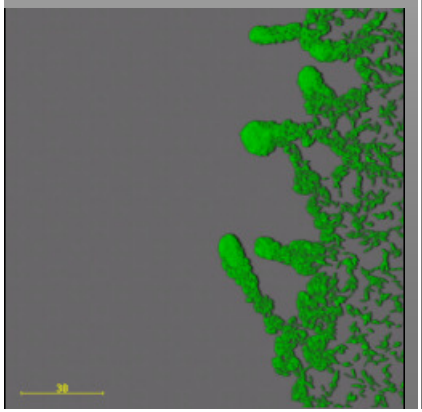
**µm scale**

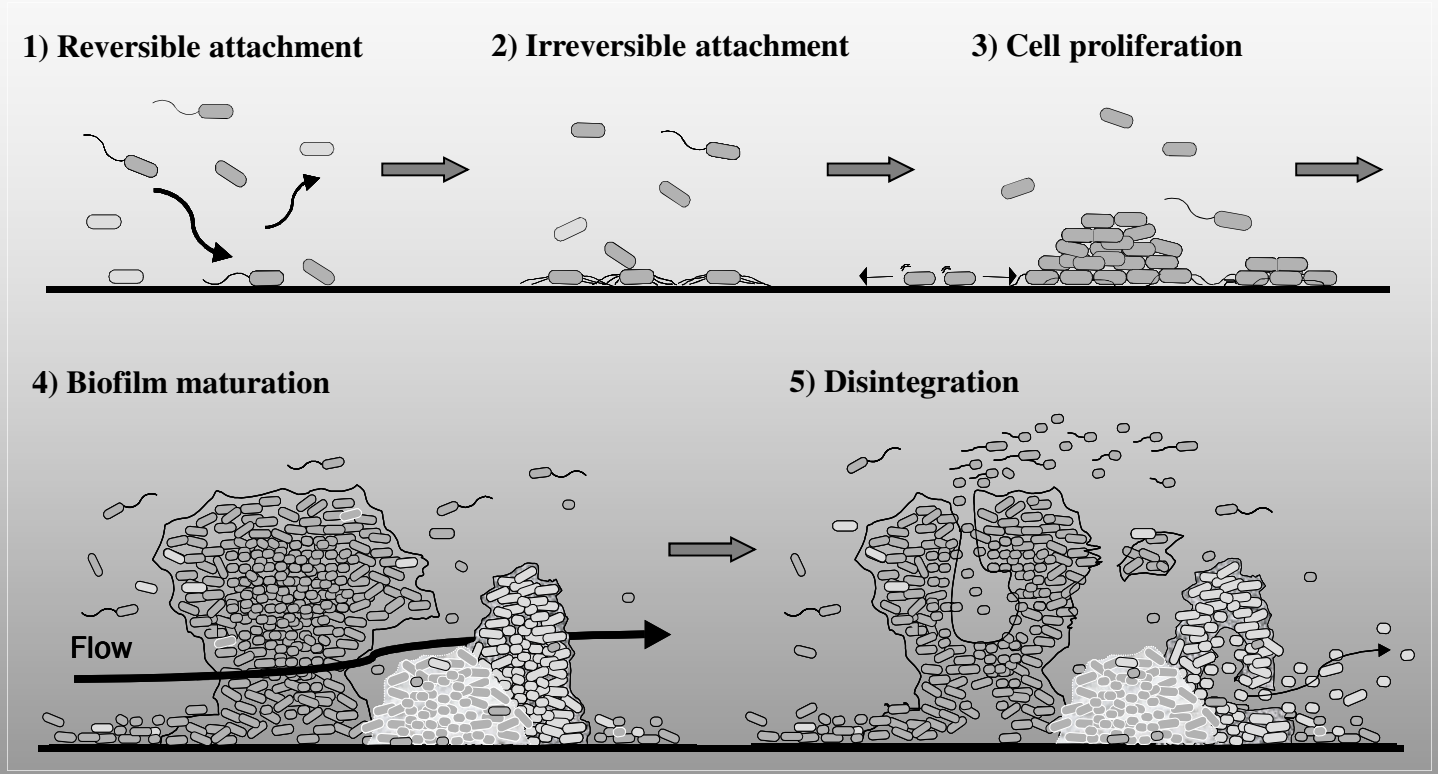


# The Biofilm Development Cycle

**In hydrodynamic conditions biofilm development depends on *adhesive forces*.**

**At the substratum bacterial motility may have significant impacts on structure development**



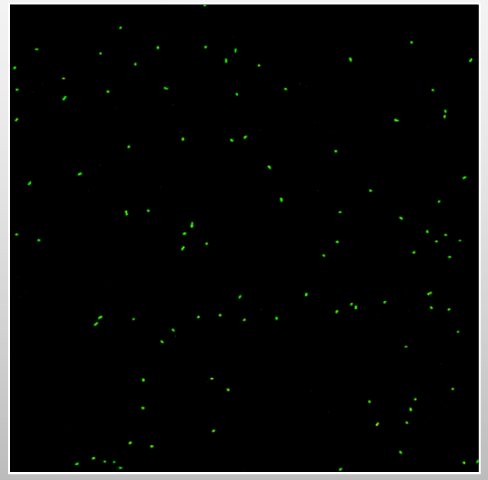



- ***Colonizers*** – able to attach to surface
- ***Stickers*** – able to form micro-colonies
- ***Twitchers*** – able to migrate on surface
- ***Swimmers*** – able to move in water column
- ***Swarmers*** – able to spread on conditioned surface

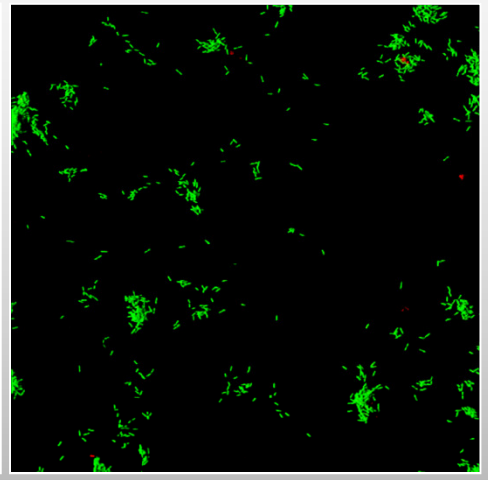
# Biofilm Development

Biofilm development as seen through the scanning confocal laser microscope

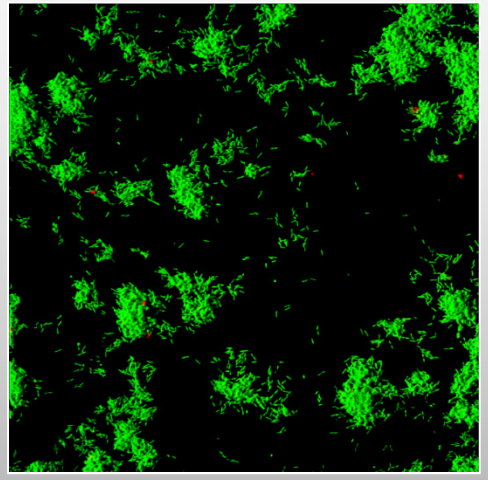
1) Reversible attachment



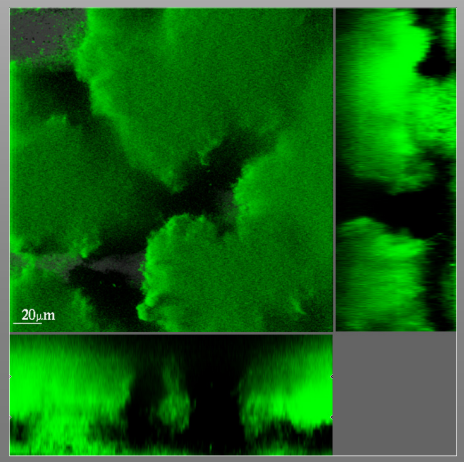
2) Irreversible attachment



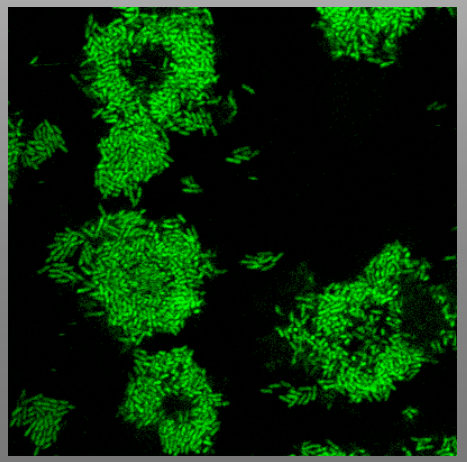
3) Cell proliferation



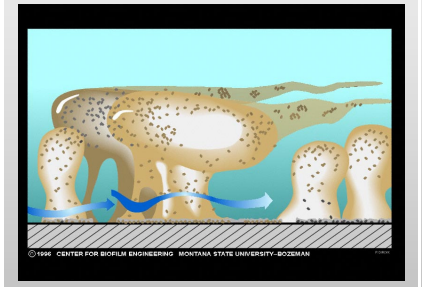
4) Biofilm maturation




5) Dissolution



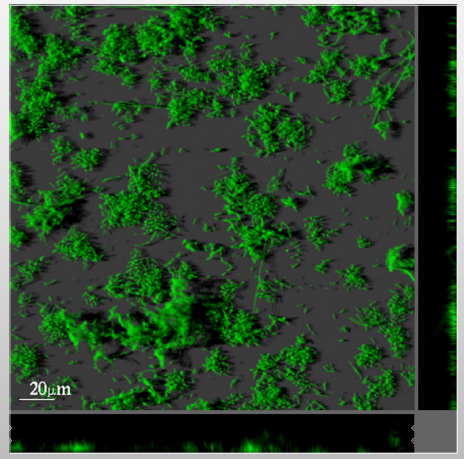
# The Zoo: Mature Flow-Chamber Biofilms



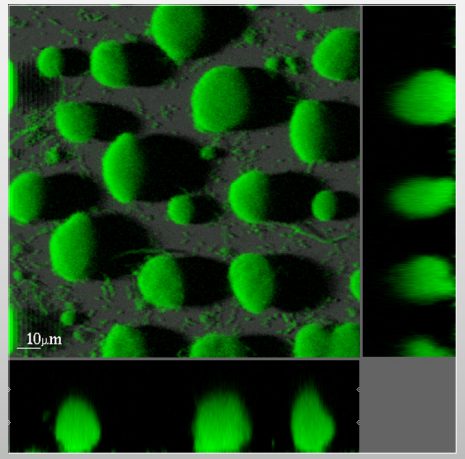

The *P. aeruginosa* model

No indication of consensus structure of mature biofilms

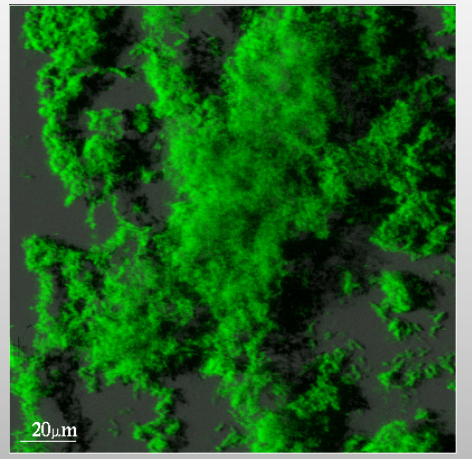
*E. coli* F-



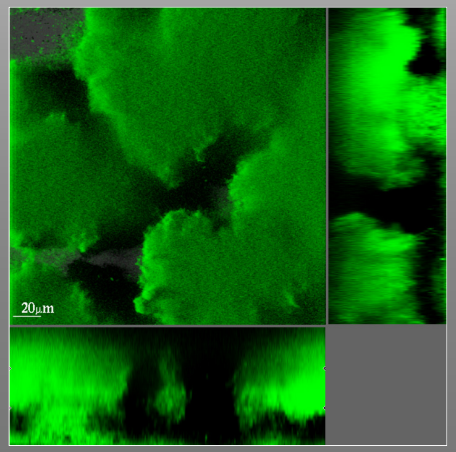

*Acinetobacter spp.*



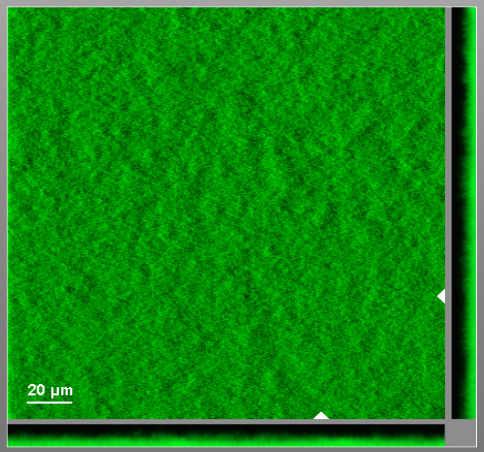

*P. putida*



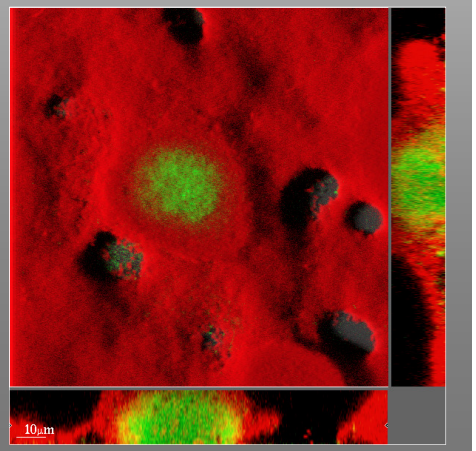

*E. coli* F+/
*P. aeruginosa* (1)




*P. aeruginosa* (2)




*P. syringae*



# Biofilm Development

**Biofilm structure/function development is influenced/determined by:**

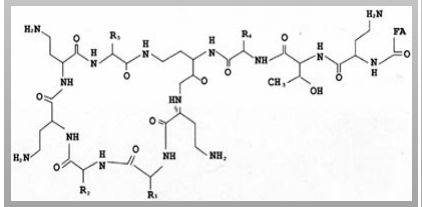
- **The nutritional conditions (cf. Cristian Picioreanu)**
- **The characteristics of cell surface adhesives**
- **The motility properties (swimming, twitching...)**
- **Development of distinct sub-populations**
- **Matrex production (Polysaccharides, proteins, ekstracellular DNA...)**
- **Cell to cell communication (quorom sensing...)**
- **Exchange of genetic information (conjugation, transduction)**

**In addition, specific dissolution features may impact the structural properties of biofilms**

**And the major factor driving biofilm development:**

**Food acquisition**

# Subpopulation development and differentiation in *E. coli* biofilms

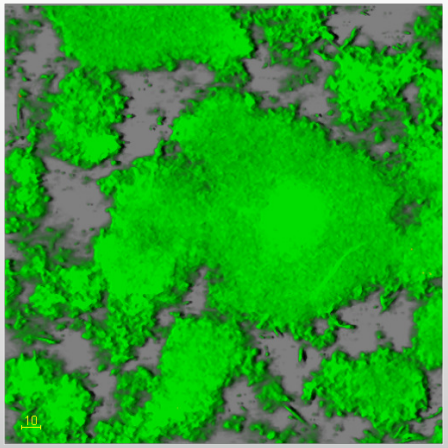


Colistin

- *E. coli* as the model organism
  - -identification of specific subpopulations by antibiotic treatment of biofilms
- Colistin (a polymyxin) as the model antibiotic
  - Mode of action: Bactericidal, binds to and interferes with the cytoplasmic membrane
- In situ monitoring of live and dead cells in biofilms by use of the BacLight Live/dead kit
- Colistin acts directly on the membrane, the target for the BacLight indicator

# Antibiotic activity in *E. coli* Biofilm

Gfp tagged micro-colony. Before treatment with colistin.

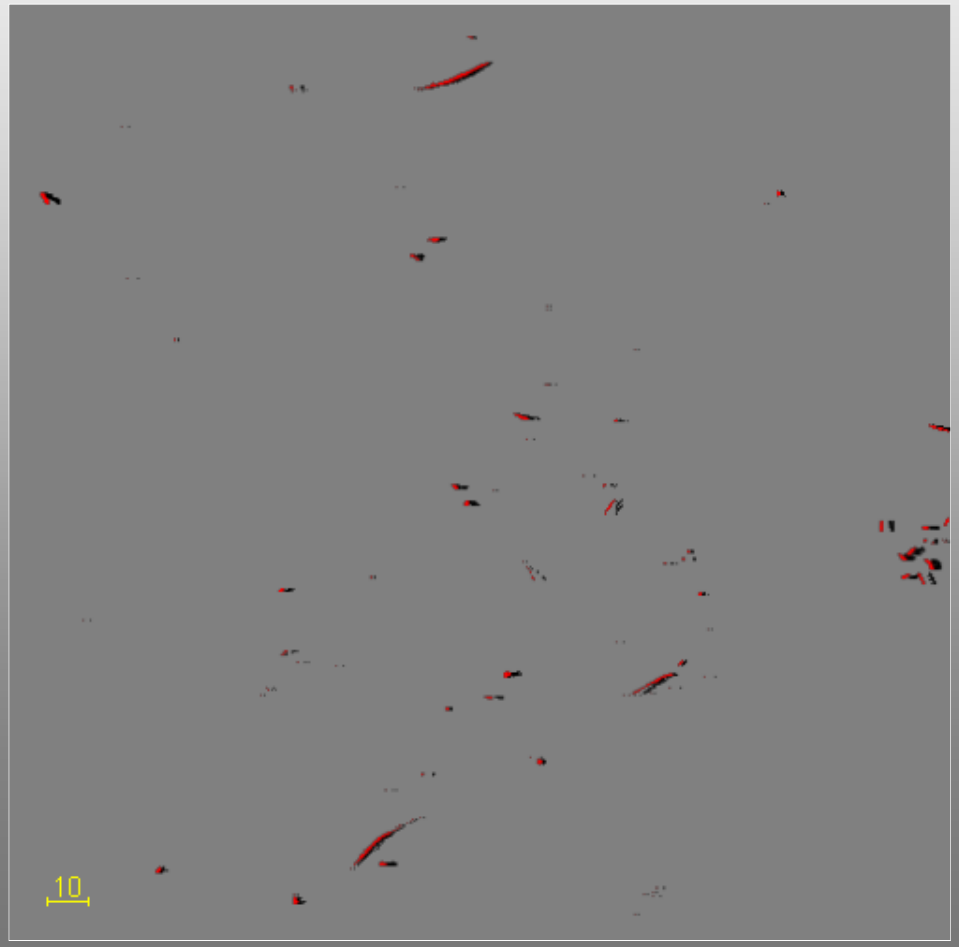



Treatment with colistin simultanius staining of dead cells with Propidium Iodide

**Green cells are alive**

**Red cells are dead**

## Dynamics of killing (20hrs)

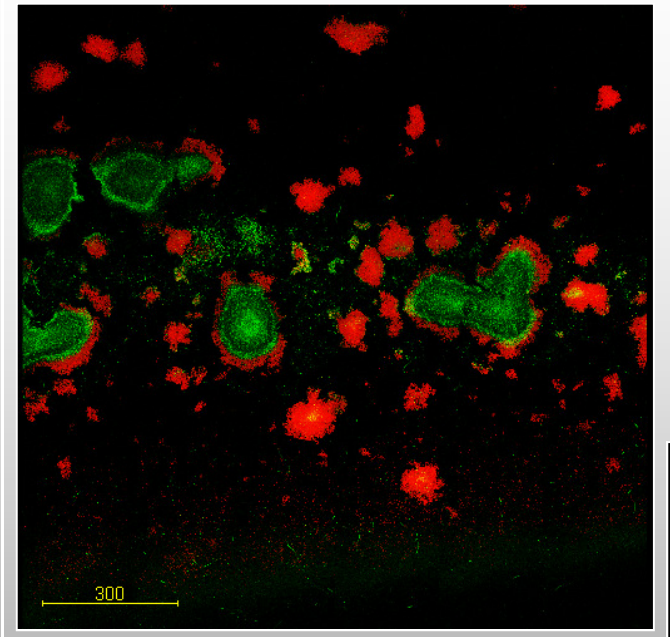



The killing activity of colistin moves from the edge of the micro-colonies to the inner parts

No detachement of dead cells

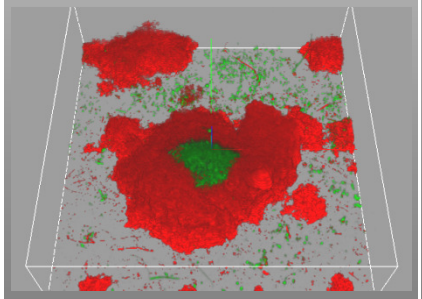
# Antibiotic tolerance in *E. coli* biofilm

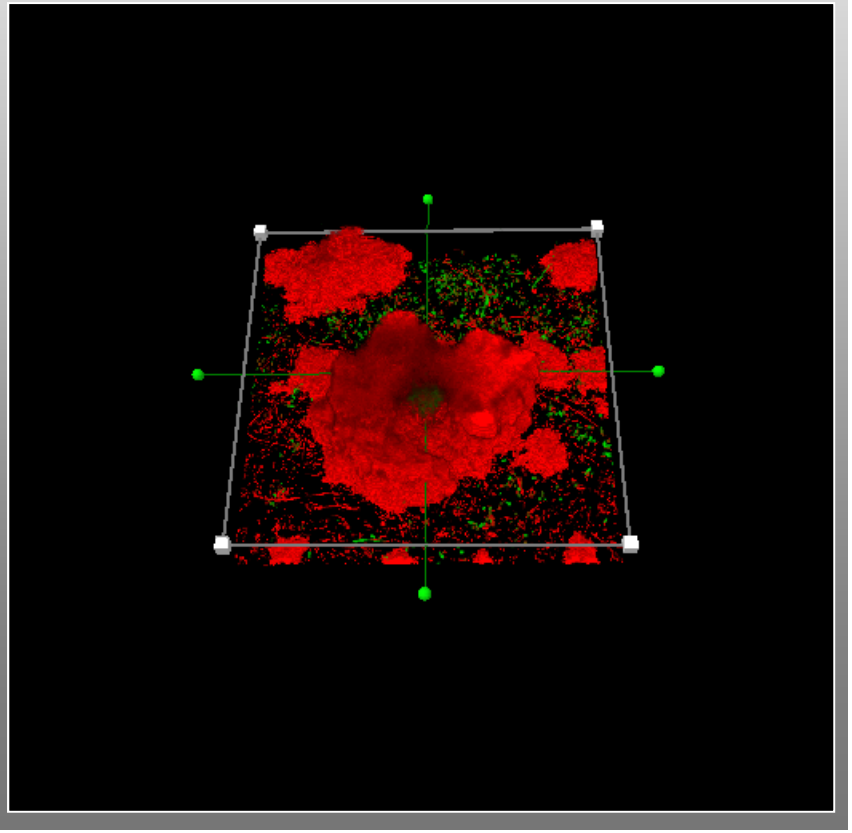
**Gfp labeled cells**




Overview of biofilm structure (10x)

Colistin treatment for 24 hrs followed by staining with propi-dium iodide





**Cell differentiation result in subpopulations with increased tolerance to antibiotic**

- Imaris, a image treatment and projection software

- Demonstration